1. An assay for trypsin inhibitors in urine which comprises (a) contacting a urine test sample with a buffered assay medium consisting essentially of (i) trypsin, (ii) a substrate for trypsin which will produce a detectable response when cleaved by trypsin and (iii) a polycarboxylic chelating agent in sufficient quantity to inhibit interference with the assay from calcium present in the urine as assay reagents, wherein calcium present in the buffered assay medium is not present in sufficient quantity to interfere with the binding of calcium present in the urine test sample with the polycarboxylic chelating agent, and (b) correlating the concentration of trypsin inhibitor with the detectable response from the cleaving of the substrate.

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REMARKS

I. STATUS OF THE CLAIMS

Claims 1-14 remain pending in the application. Claims 11-14 have been withdrawn from consideration. No claims have been allowed. Claims 1-10 remain rejected. No claims stand objected to. No claims have been cancelled. Claim 1 is amended herein.

II. SUMMARY OF THE REJECTIONS

Claims 1-4 and 7-9 remain rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over U.S. Patent No. 5,856,117 to Uenoyama et al. ("Uenoyama") in view of U.S. Patent No. 5,384,247 to Berry et al. ("Berry").

Claims 5 and 6 remain rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Uenoyama in view of Berry as applied to claims 1-4 and 7-9 above, and further in view of GB Patent No. 2,204,398 A to May et al. ("May").

Claim 10 remain rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over Uenoyama in view of Berry as applied to claims 1-4 and 7-9 above, and further in view of US Patent No. 6,130,055 to Nanbu et al. ("Nanbu").

Applicants respectfully traverse these rejections and request reconsideration.

III. SUMMARY OF THE INVENTION

The present invention is an assay for trypsin inhibitors in urine which involves contacting a urine test sample with a buffered assay medium comprising trypsin, a substrate for trypsin which will produce a detectable response when cleaved by trypsin and a polycarboxylic chelating agent in sufficient quantity to inhibit interference with the assay from calcium present in the urine test sample, and correlating the concentration of the trypsin inhibitor with the detectable response from the cleaving of the substrate.

Also included within the scope of the present invention is a dry assay device having trypsin, buffer, a trypsin substrate and a chelating agent in an absorbant carrier for detecting the presence and concentration of trypsin inhibitor in urine test samples.

IV. SUMMARY OF THE AMENDMENTS

Claim 1 is amended to recite the buffered assay medium "consisting essentially of" the recited trypsin, trypsin substrate/indicator, and polycarboxylic chelating agent. Claim 1 is also amended to recite that the "calcium ions present in the buffered assay medium are not present in sufficient quantity to interfere with the binding of calcium present in the urine test sample with the polycarboxylic chelating agent". Support for this amendment to claim 1 is found in the specification, page 10, lines 14-19. "Phosphate and carboxyl groups are common as the charged ionizable groups of buffering agents and calcium salts of these groups are not very water soluble (calcium phosphate is relatively insoluble), so they tend to precipitate from solution." Additional support is found in the specification, pages 9, line 35, to 10, line 10, and throughout the specification.

Attached hereto is a marked-up copy of the claims as amended, showing the changes made. The attached pages are captioned "APPENDIX I". No new matter is added.

V. ARGUMENTS IN SUPPORT OF PATENTABILITY

Claims 1-4 and 7-9 remain rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over U.S. Patent No. 5,856,117 to Uenoyama et al. ("Uenoyama") in view

of U.S. Patent No. 5,384,247 to Berry et al. ("Berry"). The Examiner admits that the method of Uenoyama differs from the instant invention in failing to disclose the use of a polycarboxylic chelating agent to inhibit interference of calcium present in the urine. However, the Examiner alleges that it would have been obvious to one of ordinary skill in the art to incorporate the polycarboxylic chelating agents (EDTA and EGTA) of Berry into the method of Uenoyama, because Berry shows that the use of these chelating agents provide the advantage of reducing the free concentration of interfering ions to levels where interference is no longer significant and also increase the sensitivity of the enzyme to an analyte. Applicants respectfully traverse this rejection for several reasons.

First, Applicants have amended Claim 1, as suggested by the Examiner to remove the recitation that the assay components "comprise" (Applicants than the Examiner for the suggestion in the Office Action of April 9, 2002, page 6). Claim 1 now recites that the buffered assay medium "consisting essentially of" the recited trypsin, trypsin substrate/indicator, and polycarboxylic chelating agent. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. *In re Herz*, 537 F.2d 549, 551-52, 190 USPQ 461, 463 (CCPA 1976); MPEP § 2111.03. Claim 1 now also recites that the "calcium ions present in the buffered assay medium are not present in sufficient quantity to interfere with the binding of calcium present in the urine test sample with the polycarboxylic chelating agent".

Accordingly, and as shown in the Amendment of January 31, 2002, page 3, the method of the invention is clearly exclusive of the Uenoyama method, which necessarily uses excess calcium to reduce the interference of excess calcium ions already present in the urine sample, by using the additional calcium ions to swamp out its effect. Moreover, and as previously stated, "in the present invention, the addition of additional calcium is not necessary and is even detrimental since in the method of Ueonoyma et al., the calcium interferemce is not removed but only offset." Applicants note that Claim 1 continues to recite that the polycarboxylic chelating agent is present to "inhibit interference with the assay from calcium". Thus, the Uenoyama reference does not render obvious the claimed "subject matter as a whole". 35 U.S.C. § 103.

Second, Applicants submit that the Berry reference should not be combined with the Uenoyama reference. As the issue is whether the Berry reference provides the use of a reagent to selectively remove the interferent in an assay for measuring trypsin inhibitor, the use of the selective reagent being missing in the Uenoyama reference

The Examiner alleges, in reply to the Amendment of January 31, 2002, page 4, that Berry discloses the chelation of not only sodium ions but also calcium ions. Applicants reply that that Berry uses a sodium sensitive enzyme and a sodium sensitive ion which binds to sodium ions, the concentration of which is being determined. Berry does not disclose the use of a selective reagent to selectively remove the interferent. Insofar as Berry uses a reagent that is not selective for the interferent, Berry does not render obvious the claimed invention.

Indeed, the specification clearly shows that calcium and sodium are not equivalent for this invention. The specification, pages 9, line 35, to 10, line 10, states that calcium is an inhibitor of trypsin and "Analysis of other urinary components such as other salts, specific gravity and pH did not demonstrate correlation between the expected and observed results. The testing of a number of combinations of potential urine trypsin activators and inhibitors was carried out with the result that calcium was determined to increase their activity while chloride, sodium and magnesium were found to have little effect. It was further determined that the calcium either had to be overwhelmed or complexed to remove it from the assay system. Since the long term goal is to produce a dry phase test for urinary trypsin inhibitors, and the calcium would precipitate most buffers, it was decided to try to remove the calcium by complexing."

Third, the Examiner alleges, in reply to the Amendment of January 31, 2002, page 4, that Berry discloses the chelation of ions for transferase, hydrolase, oxidoreductase, and lyase, and further that trypsin is a protease. Applicants reply that prior knowledge of general methods of inhibiting broad classes of enzymes does not by itself render obvious the specific and selected assay for measuring trypsin inhibitor. MPEP § 2144.08; Compare to *In re Baird*, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994) (discussing the size of the genus.). Applicants also submit that the principle of operation in the present invention (selective binding of an interferent) is

different from the purpose of Berry's ion chelation. Accordingly, the Berry reference, even in combination with the Uenoyama reference, does not render obvious the claimed "subject matter as a whole". 35 U.S.C. § 103.

Fourth, even if the Uenoyama reference and the Berry reference were combined, the combination would not have been reasonably expected to result in the claimed invention. As stated in the specification, page 4, lines 21-30. "The assay of the present invention is based on the discovery that the interference with the urine trypsin assay caused by the presence of calcium ion in urine can be factored out of the assay by the use of certain chelating agents. This was unexpected because the chelating agents were not used to extract and remove calcium but only to complex the salt. It was to be expected that trypsin would still interact with the complexed salt in a detrimental fashion."

In summary, the claimed invention, as amended, is not obvious in view of Uenoyama and Berry. Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103.

Claims 5 and 6 remain rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Uenoyama in view of Berry as applied to claims 1-4 and 7-9 above, and further in view of GB Patent No. 2,204,398 A to May et al. ("May"). The Examiner admits that Ueoyama differs from the instant invention failing to disclose dry test reagents and a dry test device which the urine test sample can flow by dipping the dry test device into the buffered assay medium. However, the Examiner alleges that it would have been obvious to one of ordinary skill in the art to use the device of May to practice the method of Uenoyama as modified by Berry, because May shows that the device allows for quick and convenient use and requires the user to perform as few actions as possible, where all the necessary reagents are present on a single solid support. Applicants respectfully traverse this rejection.

The combination of Uenoyama and Berry do not render obvious the claimed invention in a wet form and the addition of May does not render obvious the claimed invention in a dry form. Moreover, Applicants have noted above that the specification

pages 9, line 35, to 10, line 10, discusses the particular advantage of the invention for dry phase test for urinary trypsin inhibitors regarding complexing the interferent, as opposed to overwhelming the interferent.

Claim 10 remains rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Uenoyama in view of Berry as applied to claims 1-4 and 7-9 above, and further in view of US Patent No. 6,130,055 to Nanbu et al. ("Nanbu"). The Examiner admits that Uenoyama differs from the instant invention in failing to disclose arginine or lysine derivatives as the substrate for trypsin. However, the Examiner alleges that it would have been obvious to one of ordinary skill in the art to incorporate the trypsin substrates of Nanbu into the method of Uenoyama as modified by Berry, because Nanbu shows that the use of the L-type amino acid residues allows for excellent solubility (col 2, line 23). Applicants respectfully traverse this rejection, since Claim 10 depends upon the nonobvious Claim 1.

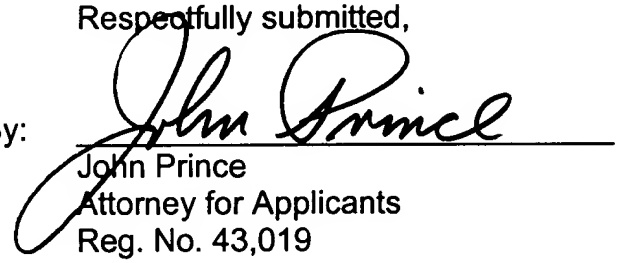
In summary, Applicants respectfully request withdrawal of all the rejections under 35 U.S.C. § 103.

VI. CONCLUSION

These amendments and remarks remove all grounds for rejection. Applicants request allowance of the remaining pending claims.

Applicants' attorney invites the Examiner to telephone if he has any questions about the application or this submission.

Respectfully submitted,

By: 

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APPENDIX I

1. (Twice amended) An assay for trypsin inhibitors in urine which comprises (a) contacting a urine test sample with a buffered assay medium ~~comprising~~ consisting essentially of (i) trypsin, (ii) a substrate for trypsin which will produce a detectable response when cleaved by trypsin and (iii) a polycarboxylic chelating agent in sufficient quantity to inhibit interference with the assay from calcium present in the urine as assay reagents, wherein calcium present in the buffered assay medium is not present in sufficient quantity to interfere with the binding of calcium present in the urine test sample with the polycarboxylic chelating agent, and (b) correlating the concentration of trypsin inhibitor with the detectable response from the cleaving of the substrate.

2. The assay of Claim 1 wherein the assay reagents are in solution.

3. The assay of Claim 2 wherein the solvent used to form the solution is an aqueous or polar aprotic solvent.

4. The assay of Claim 3 wherein the solvent is water, ethanol, methanol, isopropanol, acetonitrile, dimethyl sulfoxide, acetone, dimethylformamide or methylethylketone.

5. (Once amended) The assay of Claim 1 wherein the assay reagents are in a dry phase.

6. The assay of Claim 5 wherein the assay reagents are impregnated into a dry test device of a material through which the urine test sample can flow by dipping the dry test device into the buffered assay medium with subsequent drying of the solvent.

7. The assay of Claim 1 wherein the chelating agent is ethylene glycol bis (β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA); ethylenediaminetrata acetic acid (EDTA); iminodiacetic acid (IDA); nitrilotriacetic acid (NTA); diethylenetriaminipentaacetic acid (DTPA); triethylenetriamine-hexa-acetic acid (TTHA); 2,3-propylenediamino-tetra-acetic acid (UEDTA) and 1,2-diaminocyclohexanetetra-acetic acid.

8. The assay of Claim 1 wherein the trypsin is present in an amount of from 10 to 750 IU/mL, the chelating agent is present in an amount of from 0.2 to 50 mM, the trypsin substrate is present in a concentration of from 0.2 to 50 mM and the pH is buffered at a level of from 6.0 to 8.0.

9. The assay of Claim 8 wherein the trypsin concentration is from 100 to 500 IU/mL, the chelating agent is present in a concentration of from 10 to 25 mM, and the pH is at a level of from 7.0 to 8.0.

10. The method of Claim 1 wherein the substrate for trypsin is selected from the group consisting of arginine or lysine derivatives of 7-amino-4-methylcourmarin, 2-aminonaphthalene, 4-methoxy-2-amino-naphthalene, 3-carboxy-4-hydroxy-analine, 2-chloro-4-nitro-analine, 3-aminoindole, 2-aminoacridone, 2-aminobenzothiazole, 2-aminopyrimidine, Rhodamine 110 and 6-aminoquinoline.

11. A method for preparing a test device for the determination of trypsin inhibitor in urine which comprises contacting a pad of absorbent material with an aqueous solution of trypsin and a poly carboxylic chelating agent followed by drying the strip and contacting it with a solvent solution of a substrate for trypsin with subsequent drying.

12. The method of Claim 11 wherein the solvent solution of Claim 11 contains a non-ionic polyoxyalkyl surfactant.

13. The method of Claim 12 wherein the surfactant contains ethylene glycol units.

14. The method of Claim 11 wherein the trypsin substrate is 3-(Nα-tosyl-N_G-nitro-L-arginyloxy)-5-phenylpyrrole.